Immunity
Previews

A FAScinating Receptor in Self-Tolerance

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In this issue of *Immunity*, Stranges et al. (2007) ablate expression of the death receptor Fas in T cells, B cells, and DC. Fas deficiency in any of these lineages is sufficient to break self-tolerance.

Ever since the pivotal discoveries that mutations in the tumor necrosis factor (TNF)-family receptor Fas (also known as CD95 or Apo-1) cause the recessive autoimmue *lpr* syndrome in mice and the autosomal dominant autoimmune lymphoproliferative syndrome (ALPS) in humans (Fisher et al., 1995; Watanabe-Fukunaga et al., 1992), understanding how Fas protects from systemic autoimmunity has been the subject of intense investigation. Although Fas is well known to trigger cell death in vitro in multiple cell types, it has been less clear in which cells Fas functions to protect from autoimmunity in the intact immune system. By using mouse lines in which Fas expression is ablated in specific cell types through Cre-lox-mediated gene excision, Stranges et al. (2007) in this issue of *Immunity* offer the startling answer that autoimmunity can result from the lineage-specific loss of Fas in either T cells, B cells, or dendritic cells (DCs). This study also reveals interesting differences between the consequences of Fas deletion in each lineage.

Fas is inducibly expressed in multiple lineages of immune cells, as well as constitutively in other tissues, such as hepatocytes, whereas expression of its ligand, FasL, is more tightly controlled. FasL appears transiently on T cells only after stimulation though the T cell receptor (TCR), and in vitro, it can trigger apoptosis after TCR restimulation of activated $CD4^+$ T cells (Figure 1; Siegel et al., 2000). Early evidence from chimeric mice suggested that collaboration between Fas-deficient T and B cells is required for maximal autoimmunity (Sobel et al., 1994). Also, re-expression of Fas on T cells in Fas-deficient mice reduces autoantibody titers and loss of the "double-negative" $CD4^-CD8^-$ T cell population that characteristically develops in mice or humans with functional Fas or FasL deficiency.

Understanding the role of Fas in the intact immune system became more complex because of recent findings that have suggested alternative functions for Fas-FasL signaling. First, caspases have been recognized to have nonapoptotic cellular functions, including involvement in the activation of the NF-κB transcription factor. For this reason, blockade of Fas signaling downstream of the receptor through genetic ablation or mutation of the downstream signaling proteins FADD or Caspase-8 does not reproduce the phenotype of Fas deficiency, but instead results in defective lymphocyte development and activation (Siegel, 2006). Although interesting, these findings prevented insights from being gained about the consequences of tissue-specific Fas deficiency. The first group to employ Cre-lox-mediated tissue-specific elimination of Fas found, like Stranges et al. (2007), that Fas deletion in B cells did result in autoantibody production and lymphocyte expansion. However, T cell-specific deletion of Fas resulted in lymphocyte depletion and fatal fibrotic lung disease because of hyperexpression of FasL

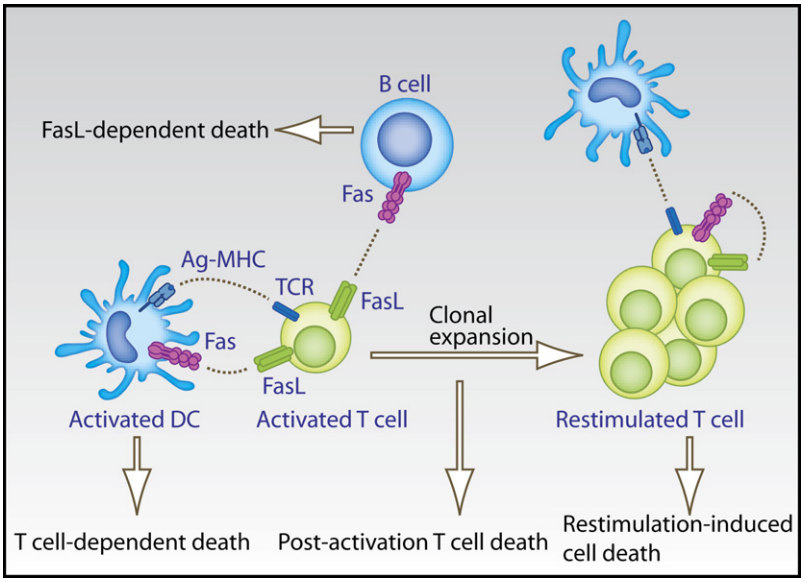

Figure 1. Fas-Dependent and -Independent Interactions that Lead to Immune Cell Death
After initial expansion, many T cells die via a passive process of post-activation cell death, which depends on the intrinsic cell-death pathways and molecules such as Bim, but not death receptors such as Fas. Fas-FasL interactions are responsible for autocrine apoptosis of restimulated T cells (restimulation cell death or RICD). FasL produced by recently activated T cells can induce apoptosis in DCs through Fas. Fas on B cells also controls B cell numbers and prevents autoantibody secretion. Deficiency of Fas in any one of these lineages as shown by Stranges et al. (2007) leads to ANA production and accumulation of different cell types depending on which lineage is rendered Fas deficient.

on Fas-deficient T cells (Hao et al., 2004). This syndrome, reminiscent of the FasL-dependent graft-versus-host disease-like syndrome that results when Fas-deficient cells are transferred into irradiated hosts, also prevented analysis of the role of Fas in T cells in otherwise healthy mice.

In this context, the results of Stranges et al. (2007) provide welcome clarification of the role of Fas in various immune cell lineages and re-emphasize the role of Fas as an enforcer of self-tolerance in multiple lineages of immune cells. By using lineage-specific expression of the Cre recombinase crossed to mice in which exon 9 of Fas was surrounded by loxP sites, Stranges et al. (2007) analyzed the fate of mice in which DCs, T cells, and B cells have been rendered Fas deficient. Previous work had suggested that Fas on DCs could be a negative regulator of T cell responses, and labeled DCs were found to have a short half-life in vivo in the presence of antigen-specific T cells. Whether or not these phenomena were due to Fas-FasL interactions was not known. From experiments mixing Fas-deficient dendritic cells and FasL-deficient antigen-specific T cells, Stranges et al. (2007) now show that FasL on T cells interacting with Fas on DC is required for antigen-specific DC deletion. When Fas was eliminated through Cre-mediated deletion, there was also accumulation of activated DCs in the spleens of these mice. More importantly, these mice developed classical markers of systemic autoimmunity including hypergammaglobulinemia and elevated amounts of antinuclear antibodies (ANAs) within 12–16 weeks of age. These results correlate well and strengthen previous observations of systemic autoimmunity in mice with DCs expressing p35, a baculovirus-derived caspase inhibitor (Chen et al., 2006). Because p35 is a blocker of multiple death receptors as well as intrinsic mechanisms of apoptosis, the study by Stranges et al. (2007) is important in confirming the specific role of Fas in DC homeostasis and controlling the immunogenicity of self-antigen presentation.

B cells have a dual role in autoimmunity both as the producers of autoantibodies and as antigen-presenting cells for autoantigens, a function that is likely enhanced in autoreactive B cells because of more efficient presentation of antigens via BCR-induced endocytosis. In the study by Stranges et al. (2007), mice with B cell-specific Fas deficiency developed marked splenomegaly and lymphadenopathy, as well as elevated ANA. Interestingly, the cells expanded in these mice were mainly T cells, suggesting that Fas on B cells is somehow necessary for restraining the accumulation of Fas-intact T cells. These results expand on earlier findings in which B cells were found to be required for the accumulation of memory-phenotype T cells in the setting of global Fas deficiency (Chan and Shlomchik, 1998).

In case of T cell-specific Fas deletion, the authors used *Lck*-Cre as well as *Gzmb* (encoding granzyme B)-Cre transgenic mice to target all T cells or activated T cells, respectively. Surprisingly, T cell-specific deletion of Fas did not lead to lymphadenopathy or splenomegaly, but these mice did produce ANAs. One feature of Fas deficiency that was only reproduced in T cell-specific Fas deficiency is the accumulation of $CD4^{-}CD8^{-}B220^{+}$ double-negative T (DN T) cells, a population of cells that develop in *lpr* and *gld* mice as well as in ALPS patients. These cells appeared in both the *Lck*-Cre as well as *Gzmb*-Cre mice, indicating that "double-negative" T cells likely arise from previously activated T cells and arise without the need for Fas deficiency in other cell types. Other groups have shown that Fas-deficient mice appear to respond normally to exogenously administered antigens. Stranges et al. (2007) confirmed this in the setting of T cell-specific Fas deficiency by demonstrating that the number of cytokine-producing antigen-specific T cells was not elevated at the peak of the primary T cell response. However, the fact that T cell-specific Fas deficiency also allowed production of ANAs suggests, at least indirectly, that Fas deficiency in T cells alone is enough to break tolerance to nuclear self-antigens.

This study may appear to conflict with previous reports that restoring Fas in one lineage of immune cells corrects many aspects of the *lpr* syndrome. Unfortunately, most of the findings in tissue-selective Fas deficiency were not directly compared with fully Fas-deficient or *lpr* mice in this study. However, the effects of Fas deficiency in different cell types are likely to be additive. True autoimmune disease itself could not be tested because this study was done with mice on the non-autoimmune-prone C57BL/6 background, in which Fas deficiency results in ANA production but little autoimmune pathology. The lack of FasL-mediated lung pathology in the T cell-specific Fas-deficient mice generated by Stranges et al. (2007) in contrast to those of Hao et al. (2004) is more puzzling, perhaps resulting from background strain differences that influence the amount of FasL hyperexpression.

The striking conclusion that emerges from these studies is that FasL-mediated elimination of DCs and B cells occurs after the presentation of both acute exposure to a foreign antigen as well as to self-antigens. However, for T cells, Fas plays a role in eliminating only those cells with a TCR specific for chronically expressed antigens including autoantigens (Figure 1). These findings mesh nicely with recently described clinical observations of patients who have developed ALPS from somatic dominant-negative Fas mutations detected only in the T cell lineage (Holzelova et al., 2004). Investigating the complex crosstalk between different immune cell lineages when Fas is absent will undoubtedly result in more fascinating findings about this receptor that is so critical in self-tolerance.

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T Cells Doing It for Themselves: TGF-β Regulation of Th1 and Th17 Cells

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The source of TGF-β, which regulates inflammation in various disease settings, is unclear. In this issue of *Immunity*, Li et al. (2007) find that not only does T cell-produced TGF-β regulate Th1-cell-mediated inflammation, but it is also required for the generation of Th17 cells in vivo.

Transforming growth factor-β (TGF-β) has long been recognized for its immunoregulatory functions in controlling inflammation. Mice carrying a *Tgfb1* null mutation suffer severe inflammatory disease leading to early death; this disease phenotype is partly associated with the dependence of Foxp3$^+$ regulatory T (Treg) cells on TGF-β signaling for their maintenance in the periphery. TGF-β production by Treg cells has also been suggested as an important (if controversial) mechanism of suppression. Recently, TGF-β has been in the spotlight because of its emerging roles in the differentiation of both inducible Foxp3$^+$ Treg cells and RORγt$^+$ T helper 17 (Th17) cells from naive T cells (Bettelli et al., 2006; Ivanov et al., 2006), with interleukin-2 (IL-2) and IL-6 acting as switch factors between Treg and Th17 cells, respectively (Bettelli et al., 2006; Laurence et al., 2007) (Figure 1). The potential cellular sources of TGF-β in vivo are many, and in the current issue of *Immunity*, Li et al. (2007) have used mice with a CD4$^+$ T cell-specific deletion of TGF-β1 to investigate the contribution of T cell-produced TGF-β to the maintenance of tolerance and development of autoreactive T cells in vivo.

Li et al. (2007) find that mice with T cell-specific deletion of TGF-β1 developed a similar wasting disease to that observed in TGF-β1 complete-knockout mice and T cell-restricted TGF-βRII knockout mice: multiorgan leukocyte infiltration, autoantibody production, and early lethality. However, because the phenotype is less severe and disease onset substantially delayed, T cells are not the only source of TGF-β that regulates peripheral tolerance.

Unlike TGF-β1-deficient mice, the numbers of peripheral Treg cells are normal or increased in mice with a T cell-specific deletion of TGF-β. The inflammatory disease manifested in these mice therefore could not be attributed to a lack of Treg cells per se. However, it was unclear whether the disease regulation was mediated by Foxp3$^+$ Treg cells or naive Foxp3$^-$ T cells because both were observed to produce TGF-β after T cell-receptor ligation. Thus, the authors investigated the relative contributions of Foxp3$^+$ and Foxp3$^-$ cells in the T cell transfer model of colitis, in which Treg-cell-mediated suppression of inflammation is dependent on functional TGF-βRII signaling in naive T cells. In this model, Treg-cell production of paracrine TGF-β was found to be crucial for control of colitis, whereas naive T cells provide a very minor contribution. Previous studies had purified Treg cells on the basis of CD25 expression—also a marker of activated T cells—which may have confounded the results. The distinct advantage of the current study was the purification of Treg cells on the basis of Foxp3 expression, thus eliminating contamination from activated T cells and clearly demonstrating an essential role for Treg cell production of TGF-β.

One of the most striking findings of this study is the clear dichotomy in the generation of effector T helper subsets: T cell-specific deletion of TGF-β resulted in increased frequencies of Th1 and Th2 cells but a decrease in the frequency of Th17 cells. This revealed two important points. First, the multiorgan inflammatory disease and colitis observed in these mice are predominantly mediated by Th1 cells and do not require the generation of Th17 cells. Second, T cells themselves are